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MEA Application Note:
Preparation of Hippocampal Slices for Organotypic Cultures (OTC)

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1 Introduction

1.1 About this Application Note

The intention of the MEA Application Notes is to show users how to set up real experiments with the MEA System on the basis of typical applications that are used worldwide.

The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

This application note includes a complete protocol for the dissection of rat brain, the preparation of brain hippocampal slices for acute and long term experiments, suggestions for long-term cultures, suggestions for MEA System configurations, and references.

1.2 Acknowledgement

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us. A major part of this document is based on the instructions provided by Dr. Andrea van Bergen and by the laboratory of Dr.Ulrich Egert.

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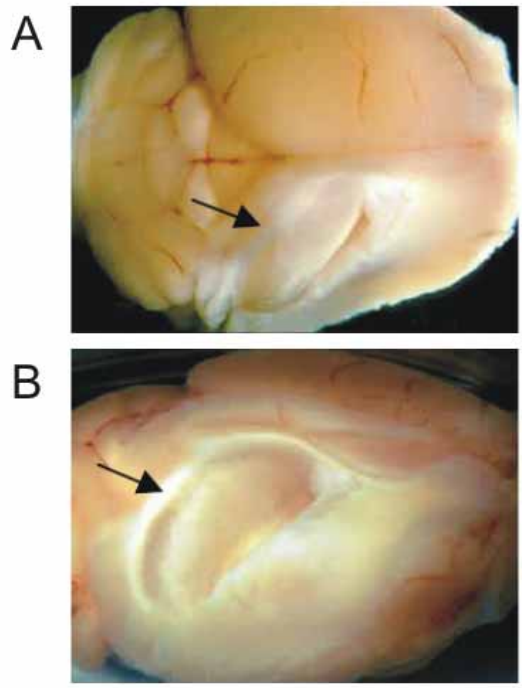
2 Aim of the Experiment

Electrophysiological experiments on brain slices are established since about 40 years, and have the main advantage that the brain tissue is more accessible in a slice than *in vivo*. Brain slices give the experimenter the opportunity to study a layer of cells organized according to their original spatial relationship with excellent preservation of cell structure, and are therefore a very powerful object of study.

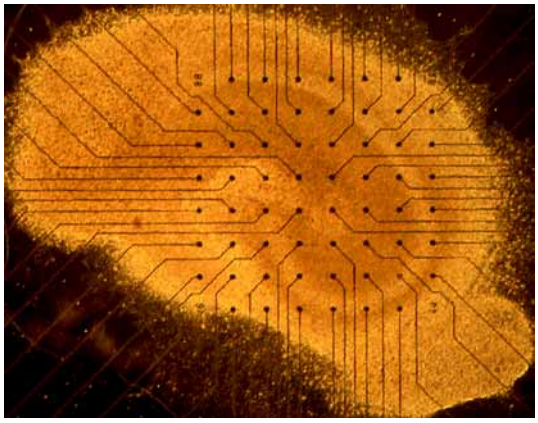
The microelectrode array (MEA) technology has further advanced the use of brain slices. The availability of MEAs — combined culture dishes and recording chambers with integrated microelectrodes — and multichannel amplifiers with an integrated temperature control allow the experimenter to exactly control the environmental conditions for the slice, greatly increasing the reproducibility of experiments. The two-dimensional electrode pattern makes it possible to study not only the temporal, but also the spatial distribution of the electrophysiological activity.



A View on a mouse hippocampus. The right hemisphere of the neocortex was removed.
B Close up view from the side.

Any number of the 60 electrodes can be used for an electrical stimulation of the tissue, for example for longterm potentiation (LTP) or depression (LTD), or paired pulse facilitation experiments.

Drugs can be applied either by the perfusion system to the entire slice, or as a microdrop application to selected regions. The drug can then be washed out after recording the response to study the reversibility of the drug effects. This extends the use of this assay from the basic research to the pharmaceutical industry as well.



Hippocampus slice mounted on an MEA.

Hippocampus brain slices are the most frequently used slice type in electrophysiology, because the hippocampal structure is easily identified in the brain, and also because its main organotypical characteristics are preserved in the slice and also in culture. Of course, it is also possible to record from other brain areas such as cerebellum or cortex. This application note focuses on the hippocampus preparation from rat.

In an acute experiment, the slice (generally from adult animals) is used directly after preparation and discarded after the experiment. Slices from neonatal animals can also be kept in prolonged culture, over several weeks or even months, as so called organotypic cultures (OTC). Even though the synaptic organization is not exactly the same as in native tissue, the main characteristics and functions are preserved. OTCs have the advantage that they allow to observe the electrophysiological activity over a longer period of time on the same slice. Also, organotypic slices tend to thin out on the MEA, which is advantageous for optical imaging.

OTC experiments are especially useful for longterm experiments, for example, for monitoring the development of the neuronal networks and its electrophysiological activitiy, the behavior of cocultured slices from different regions and the regeneration of tissue, or longterm drug effects.

3 Material

3.1 Biological Materials

- Neonatal rat (for example Wistar Kyoto, Sprague Dawley, or Long Evans, P1–P7). Older rats may be used for acute experiments, but the tissue is generally not viable enough for longer culturing. It is important that the animals are not prepared directly after a stress situation, for example, transportation, as this may impair the quality of the tissue.

3.2 Technical Equipment

- MEA System (with amplifier and data acquisition, see Suggested MEA System)
- MEAs (microelectrode arrays)
- Stimulus generator
- Peristaltic pump
- Stereo microscope
- Inverted microscope (Necessary for aligning the electrode positions to the slice. If you prefer to use an upright microscope, you need, for example, a camera and a stereo microscope for documenting the electrode position. A picture of the slice on the electrode field can then be loaded into the MC_Rack program for aligning the data traces to the electrodes.)
- Incubator for example, Heraeus Cytoperm from Kendro Laboratory Products, www.kendro.com, set to 35 °C, (5 % CO_2), adapted with a tilt mechanism for culturing slices. Degree of tilt and speed: –70°/+70° per minute, with a resting time of 2 min in-between rotations.
- Ice
- Cooled metal block
- Tissue chopper (for example, McIlwain from Campden Instruments Ltd.) or oscillating microtome (for example, Integraslice from Campden Instruments Ltd.) and blades
- Adjustable pipettes and pipette tips (20 µL and 1000 µL)
- Large sharp scissors or guillotine
- Surgical instruments, for example a bone rongeur and sharp scissors
- Narrow flat spatula for removing the brain
- Sharp forceps
- Curved forceps
- Small scissors
- Razor blade
- Sharp pointed spatula with very smooth surface, for separating the slices: Sharpen and sand smooth the spatula thoroughly before each use.
- Filter paper tips (folded or thick filter paper cut in triangular shape)

3.3 Chemicals

- Carbogen gas (95 % O_2, 5 % CO_2)
- Chicken plasma (Gibco/Invitrogen)
- Thrombin (50 U / mL, Sigma-Aldrich, Inc.)
- Gey's Salt Solution (Laboratoires EUROBIO, CS1GEY00)
- Glucose D (+) (Merck KGaA,108337)
- Kynurenic acid (Sigma-Aldrich, Inc., K3375)
- HCl
- Super glue (cyanoacrylate)
- 100 % alcohol or acetone (for cleaning the MEA contact pads)
- Basal Medium Eagle, contains Earle's salts, but no L-glutamine. (Gibco/Invitrogen)
- Hanks' Balanced Salt Solution (HBSS) without Calcium / Magnesium (Gibco/Invitrogen)
- Horse serum (Donor Equine Serum from HyClone)
- L- Glutamine (Gibco/Invitrogen)

3.4 Media

3.4.1 Dissection Buffer

Gey's Salt Solution

Glucose D (+) 0.5 % (w/v)

Kynurenic acid 1 mM

→ Mix and adjust pH to 7.2 with HCl. Chill to 4 °C and carbogenate with 95 % O_2 / 5 % CO_2.

3.4.2 Culture Medium

Basal Eagle medium	50 % (v/v)
HBSS	25 % (v/v)
Horse serum	25 % (v/v)
D-Glucose	0.5 % (w/v)
L-Glutamine	1 mM

Store at 4 °C for up to two weeks.

4 Methods

4.1 MEA Pretreatment and Coating

MEAs should be hydrophilized to improve tissue attachment, for example with a plasma-cleaner treatment (see also MEA User Manual). For long-term cultures, MEAs should be sterilized before use, for example by autoclavation at 121 °C for 40 min.

Depending on the type of selected MEA, various coatings may be applied to the MEA surface to promote the adhesion of the slice. An approved coating method for this application is the coating with nitrocellulose.

Suggestions for the handling of MEAs and coating methods can be found in the MEA User Manual available in the Download section of the MCS web site.

After use and cleaning of the MEAs, MEAs should be stored in distilled water at 4 ° C to maintain the hydrophilic characteristics.

4.2 Setting Up the Vibratome

Using a tissue chopper is easier and cheaper than using a vibratome. Tissue choppers are especially used for rapid chopping of tissue such as hippocampus. On the other hand, vibratomes provide more precise and gentle sectioning of tissue, and therefore generate higher quality slices.

Note: As the design and handling of different vibratomes varies, please consult the manual of your vibratome for more details.

1. Fill outer vibratome chamber with ice.
2. Fill vibratome chamber with frozen dissection buffer. You might need to add some room temperature buffer.

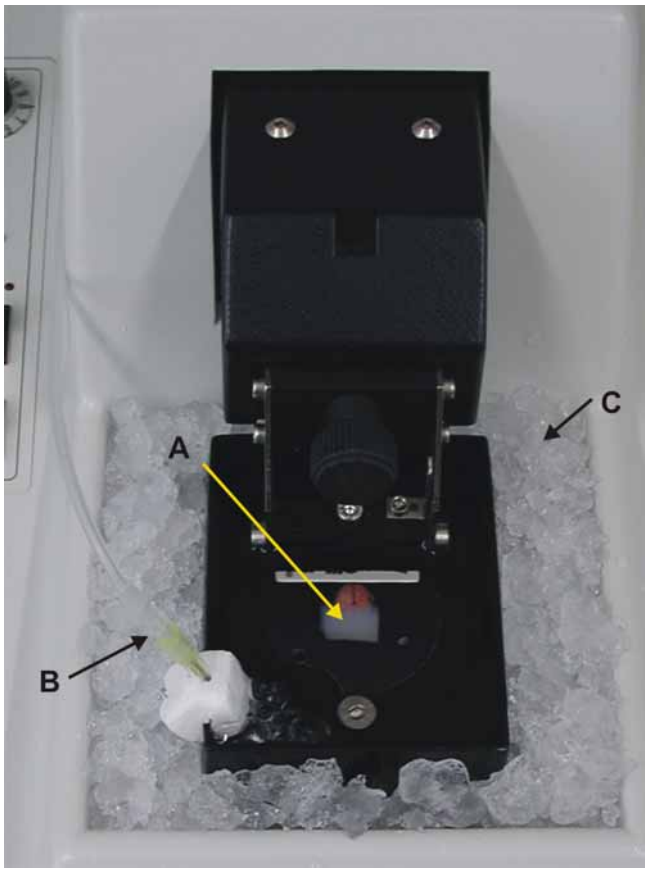


Figure 1 ***Vibratome overview:*** *A* ***Brain mounted on agar, and base plate***
B ***Oxygenation for buffer-filled inner chamber*** *C* ***Ice filled outer chamber***

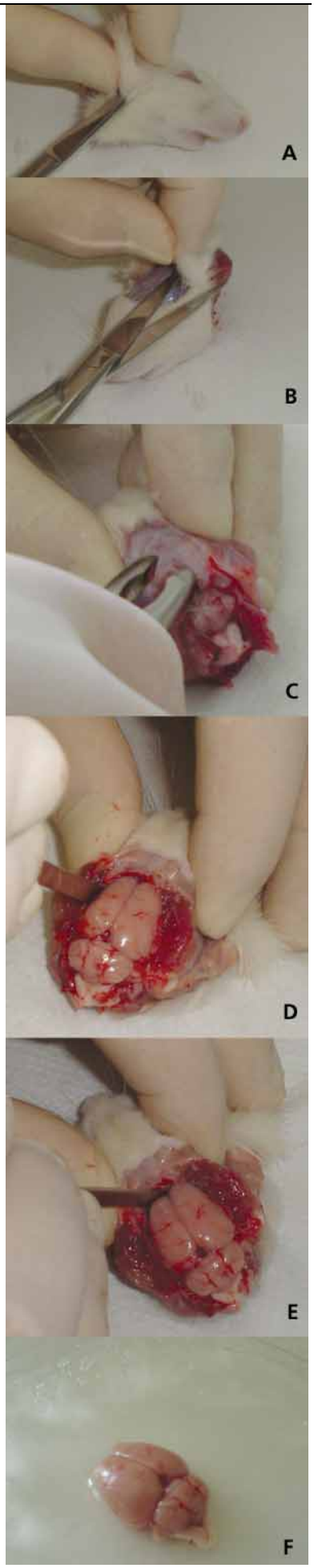
4.3 Decapitation and Brain Removal

Warning: Only qualified personnel should be allowed to perform laboratory work. Always make sure you fulfill the requirements of local regulations and laws. Work according to good laboratory practice to obtain best results and to minimize risks.

Note: The steps after step 2 should be completed within a minute or less to avoid damage of the brain. Avoid mechanical stress on the brain.

1. Decapitate the animal with large sharp scissors.
2. Cut the scalp down the midline with a scalpel, from caudal to rostral (see fig. A, B).
3. Pull down the skin along the sides of the head by grasping and pulling it between thumb and index finger from below (see fig. B).
4. Skull the cranium carefully with a fine pair of scissors on ice as follows. This method is simple and fast. It has the disadvantage that the neocortex may get damaged during the procedure. This is generally not a problem as the hippocampus is located inside the temporal lobe and should be safe.
   a. Midline all the way from foramen magnum to the front end of the brain
   b. Perpendicular to cut between forebrain and cerebellum
   c. Perpendicular to (a) along the front end of toward the base of the brain

   — OR — As an alternative method that allows obtaining an almost undamaged brain:
   Open the skull with a bone rongeur and carefully remove the skull in pieces (see fig. C).
5. Proceeding from 5c: Fold the two skull segments toward the sides.
6. Quickly scoop the brain out of the skull with the blade of a fine spatula (see fig. D, E).
7. Briefly wash the brain in a 100 mL beaker filled with ice-cold dissection buffer.
8. Place the brain in 200 mL ice-cold carbogenated dissection buffer for 10 min to quickly cool it down (see fig. F).



4.4 Dissection

The dissection should be performed both well and quickly. About 80 % of the slices should be healthy and can be used for culturing.

1. Place the brain with the dorsal side up onto a wetted filter paper in a Petri dish filled with frozen dissection buffer. The Petri dish should be kept on an ice-cold metal block to ensure a stable cool temperature.

2. Dissect the brain in the frontal plane, and discard the frontal part.

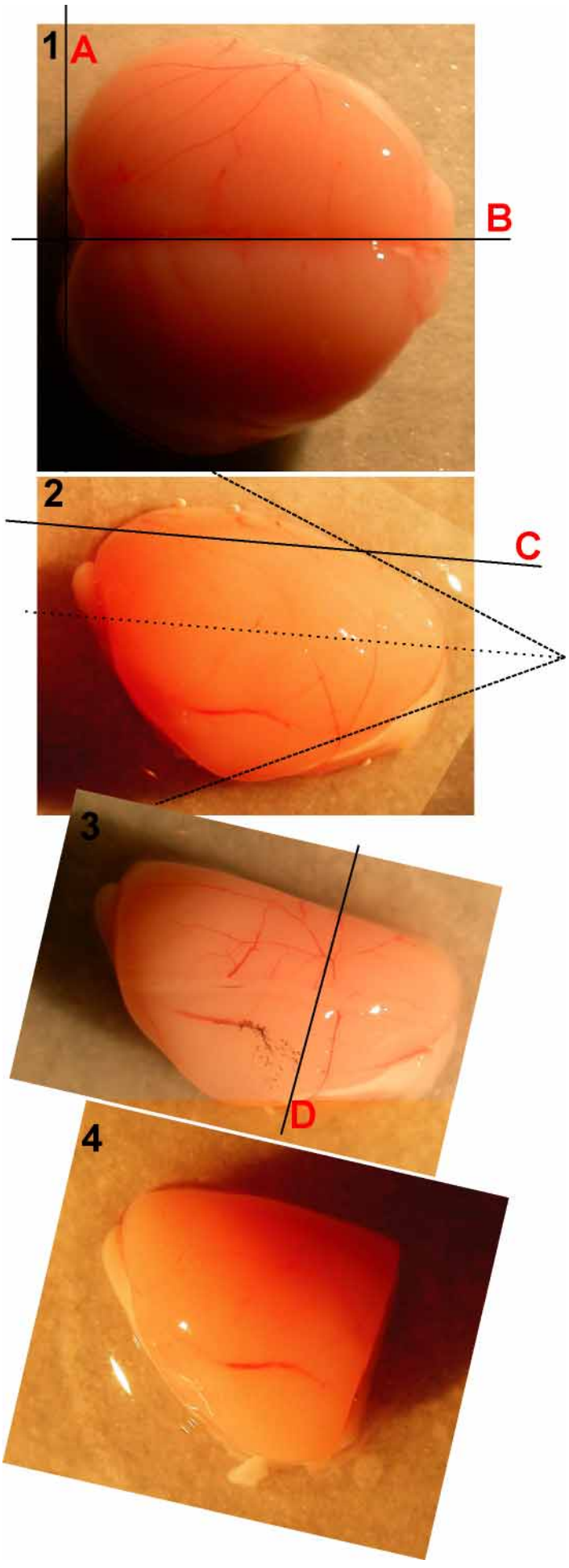
3. Cut the brain in the sagittal plane and separate the hemispheres (**cut B**, see fig. 1).

4. Remove one hemisphere and store it in cold dissection buffer until use.

5. Turn the other hemisphere onto the sagittal surface.

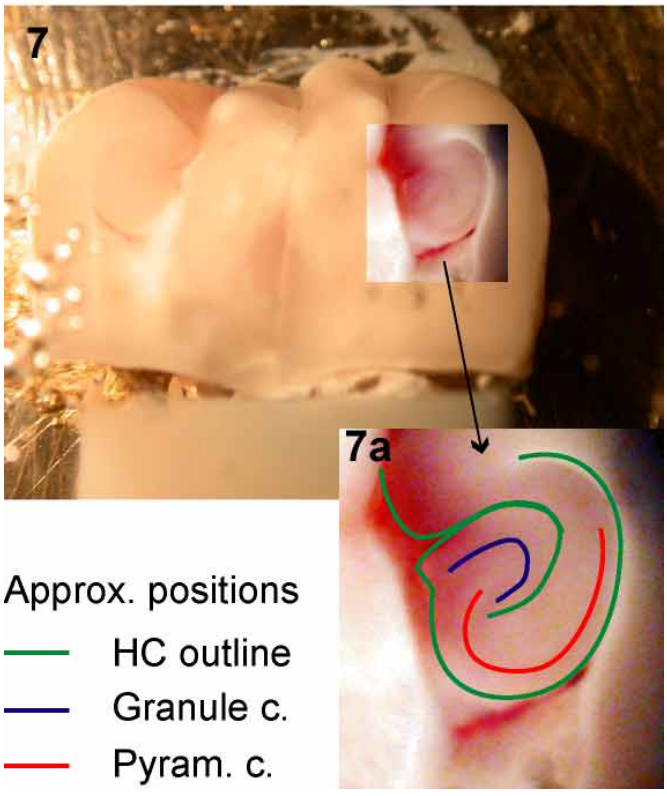
6. Cut horizontally a small planar layer from the dorsal side (fig. 2, **cut C**), in parallel to the bisecting line of the angle formed by the ventral and dorsal area of the brain (see dotted lines, fig. 2). Remove the dorsal piece, which should be less than 2 mm thick, and keep the ventral part for preparing the slices.

7. Remove one third from the rostral end from the forebrain to make the slices smaller (**cut D**, see fig. 3 and 4).



4.5 Preparing Slices

1. Glue the block of agar onto the base plate with a very small drop of superglue.
2. Place a very small drop of superglue in front of the block. Make sure the drop does not touch the agar.
3. Pick up the trimmed hemisphere with a razor blade, thus that the dorsal plane aligns with the blade.
4. Place the razor blade with the brain against the front side of the agar block and lower it towards the plate (onto the glue). The blade must not touch the glue.
5. Withdraw the blade with an upward motion. You are now looking at the ventral side of the brain (see fig. 6).
6. Place the base plate with the brain into the vibratome chamber.
7. Lubricate the razor blade with the dissection buffer to avoid that the slices stick to the blade.
8. Remove about 3 mm with the first cut — the hippocampus should become visible now (see fig. 7).
9. Slice the brain according to the recommendations of the vibratome's manufacturer. Typical hippocampal slices are about 425 µm thick.
10. Transfer the hippocampus slices very carefully to a dish filled with cooled dissection buffer. Use a lubricated spatula and avoid folding and mechanically stressing the slice.
11. Separate and sort the slices very carefully from each other with a very sharp and smooth spatula under a stereo microscope. Avoid any damage to the tissue.
12. Discard all slices that cannot fulfill the quality requirements: Unharmed tissue, slices from the middle of the hippocampus with clearly visible pyramidal and granule layers, and fimbria.



13. Store the freshly cut slices in oxygenated dissection buffer at 4 °C for 20–30 min. Some time is needed to quiet the high initial activity (due to the injuries inflicted by the slicing), but a too long time can also impair the slice quality. Prepare remaining animals in the meantime.

4.6 Mounting Slices onto MEAs

Important: Do not touch the slice directly. The slice should not be folded to avoid damage to the tissue. Be careful not to touch the MEA surface with the transfer pipette to avoid damage to the electrodes.

1. Pipette 12 µL chicken plasma onto the recording field of the MEA.
2. Carefully mount the slice onto the MEA with a broad spatula, right into the drop of plasma.
3. Distribute the drop with a pipette tip. Position the slice very carefully by gently pushing it with a pipette tip from the sides into place (see fig. A). The CA1 region should cover the recording area.
4. Pipette 12 µl thrombin onto the slice. It is not necessary to mix it with the plasma.
5. Incubate for 5–10 min to allow a rigid clot that glues the slice onto the MEA.
6. Add 1.4 mL of culture medium.
7. Cover the culture chamber with a semipermeable membrane lid.

4.7 Culturing Slices

The MEA with the mounted slice can be cultured in an incubator at 35 °C that was adapted with a tilt mechanism. Recommended degree of tilt is –70° to +70° per minute, with a resting time of two minutes in-between rotations. The tilting is necessary to ensure that the air and the medium cover the slices in alternating cycles.

Using an aeration with 5 % CO_2 is required if culture chambers with semi-permeable lid are used. If using gastight lids, no aeration is necessary. Half the volume of the culture medium should be replaced about twice a week.

Figure 2 ***Adapted Heraeus Cytoperm incubator for incubating organotypic cultures.*** *The shown incubator was adapted by the user for culturing organotypic slices on MEAs. It is possible to incubate the MEAs only and remove them from the incubator for recording, or to incubate complete MEA1060 amplifiers (see top shelf) and record inside the incubator.*

In order to allow long-term cultivation and recording without a continuous perfusion, Multi Channel Systems recommends the use of teflon membranes developed by Potter and DeMarse (2001).

The sealed MEA culture chamber with transparent semipermeable membrane is suitable for all MEAs with glass ring. A hydrophobic semipermeable foil from Dupont that is selectively permeable to gases (O_2, CO_2), but not to fluid, keeps your culture clean and sterile, preventing contaminations by airborne pathogens. It also greatly reduces evaporation and thus prevents a dry-out of the culture.

The ALA-MEA-MEM membrane is produced in license by ALA Scientific Instruments Inc., and distributed via the world-wide network of MCS distributors.

Another possibility is to use a MEA culture chamber with gastight lid (available from Multi Channel Systems), which can also be combined with a suitable perfusion system for a continuous perfusion.

4.8 Preparations for Recording

Note: We recommend the perfusion cannula with temperature control (PH01) for optimal environmental conditions. A two-channel temperature controller (TC02) allows to control both the MEA temperature (via the heating integrated into the amplifier) and the buffer temperature.

See the MC_Rack manual or online help for detailed application examples (for example, LTP recording). For connecting and programming the stimulus generator (STG), please see the respective user manual. Please see the MEA User Manual for details on stimulation amplitudes and times that are supported by the MEA electrodes. Though TiN electrodes are very stable, an unsuitable stimulation pulse will irreversibly damage the electrodes.

We highly recommend the following preparations and tests before you start the experiment. Please note that a perfusion is not be required for all experiments. Generally, you can record for **about 30 min without perfusion**. This is very convenient if you use MEAs with integrated reference electrode: You can keep the culture chamber closed and sterile during recording.

1. Test all connections.
2. Define your virtual rack specific to your application with the MC_Rack program and test it before use.
3. Define your stimulation file with the MC_Stimulus program and test it with the test model probe and with an MEA filled with recording buffer before use. It is recommended to test a range of stimulus amplitudes and locations prior to starting your actual experiment. If you are using an external stimulating electrode, the position of this electrode should be optimized in this step as well.
4. Set up the perfusion system, and test the perfusion with an old MEA. Adjust the grounding and shielding to avoid noise pickup and 50 Hz hum.
5. Set the temperature controller to 37 °C for heating the MEA culture chamber and to 32 °C for the buffer temperature (when using perfusion). (The exact temperature values may vary according to your requirements, but please take the offset between setpoint temperature and actual temperature in the bath into account. This offset varies with the experimental setup and perfusion system used.)
6. Start carbogen aeration 15 min before mounting the slice.
7. Start the perfusion 15 min before mounting the slice at a low flow rate (0.5 exchanges/min) to obtain a stable oxygenation and pH.
8. Clean the MEA contacts with a soft tissue and pure alcohol or acetone.
9. Mount the MEA with the slice onto the amplifier as described in the MEA amplifier user manual.
10. Superfuse the slice with oxygenated culture medium prewarmed at 32 °C. The buffer volume should be exchanged 3–4 times per minute. The slice is mechanically stressed by activating the perfusion and should be perfused for about **half an hour** before recording. You can also control the parameter that you want to record, and start the recording as soon as you get a stable baseline, for example, as soon as the spike rate has stabilized.

    You are now ready for recording.

5 Suggested MEA Systems

5.1 System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, different system configurations are recommended for the recording from acute hippocampus slice preparations. All systems are available for upright microscopes as well.

- **MEA60-Inv-System**: **60-channel** MEA recording system for **inverted** microscopes. The **temperature controller** TC01/ TC02 regulates the temperature of the MEA. If the experiment requires a perfusion, the applied solution can be prewarmed with **the perfusion cannula** PH01 (included in **MEA60-Inv-System-E**).
  One **MEA amplifier** allows recording of up to 60 channels from one MEA. The three additional analog inputs can be used for feeding in data generated by other systems recording in parallel, for example, for patch clamp data. The additional digital inputs can be used for synchronizing the recording with the stimulation, or with external systems. This is the standard configuration for low-throughput academic research and high flexibility for a wide range of applications.
- **MEA60-Inv-2-System-(E)**: This system operates **2 MEA amplifiers** with a 64-channel data acquisition card. It allows the recording of software-selectable 30 channels per MEA, on two MEAs in parallel.
- **MEA120-Inv-2-System-(E)** / **MEA60-Inv-4-System-(E)**: These systems are based on a **128 channel** data acquisition card and allow the simultaneous operation of **two / four amplifiers**. These systems provide a throughput suitable for both basic research and industrial applications.

5.2 Microelectrode Arrays

Available MEAs differ in electrode material, diameter, and spacing. For an overview on available MEA types please see the Multi Channel Systems web site (www.multichannelsystems.com) or contact your local retailer.

The microfold structures formed by titanium nitride (TiN) result in a large surface area that allows the design of small electrodes with a low impedance and an excellent signal to noise ratio.

For recordings from the CA1 region, a medium spatial resolution with an electrode diameter of 30 µm and a spacing of 200 µm is generally sufficient. For slice preparations from juvenile rat or adult mouse, a spacing of 100 µm can be useful for increasing the spatial resolution. The following MEAs are recommended for the recording of local field potentials and/or spikes from acute hippocampus slices.

The use of MEAs with **integrated reference electrode** is recommended, especially for experiments without perfusion, as you can keep the MEA culture chamber closed (and sterile) during recording.

- **MEA 200/30 i. r.**: Standard 8 x 8 layout, TiN electrodes for recording and stimulation, with substrate-integrated reference electrode.
- **ThinMEA 200/30 i. r.** for high-resolution imaging. ThinMEAs are only 180 µm “thick” and mounted on a robust ceramic carrier. Tracks and contact pads are made of transparent ITO.

5.3 Amplifier Specifications

Though amplifiers with custom gain and bandwidth are available, Multi Channel Systems recommends the following settings for this application.

- Lower cutoff frequency: **10 Hz**
  With an even lower value, slow signal drifts will disturb the recordings and spike detection. With 10 Hz, field potentials and spikes can be recorded in parallel. For pure spike recording, a lower cutoff of at 300 Hz would be suitable. However, you can remove local field potentials by digital filtering with the MC_Rack program later.
- Upper cutoff frequency: **3 kHz**
  Sufficient even for rapid depolarization waveforms. If only field potentials are of interest, a reduction of the upper cutoff frequency might be considered to reduce high frequency noise.
- Gain: **1200** (MEA1060) or **1100** (MEA1060-BC)

A note on **gain**: Traditional amplifiers for extracellular recordings often have 5000x or even 10000x gain switch options. A high gain, however, increases the high frequency noise level or requires a narrower filter band. Considering a 20 µV extracellular signal, we would receive 24 mV after a 1200x amplification. An AD converter with an input range set from –400mV to 400 mV will resolve the signal in increments of 0.36 µV, which will definitely provide enough information, given the noise level of such systems in general. Therefore, there is no need for higher amplifications.

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